



Climate, plant organs and species control dissolved nitrogen and phosphorus in fresh litter in a subalpine forest on the eastern Tibetan Plateau

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Abstract

• **Key message** Fresh litter contains a higher concentration of dissolved phosphorus (DP) than dissolved nitrogen (DN), which implies a more efficient DN transformation or reabsorption in the subalpine forest on the eastern Tibetan Plateau. Both DN and DP concentrations increased with the increase of mean monthly temperature, although the concentrations were also regulated by plant organs and species.

• **Context** The dissolved nitrogen (DN) and dissolved phosphorus (DP) released from fresh litter are important pathways by which total nitrogen and phosphorus are transferred from the vegetation to soil in forest ecosystems. However, few studies have paid attention to the DN and DP in fresh litter, which affects our understanding of the nitrogen and phosphorus cycles.

• **Aims** The objectives of this study were to elucidate the dynamic characteristics of the concentrations and storage of DN and DP, and to analyze how DN and DP are affected by different plant species and organs, and climate factors.

• **Methods** Fresh litter was collected in three plots in a spruce-fir forest and classified by different plant species and organs. Concentration and storage of DN and DP in fresh litter were determined and related to the climatic variables that were monthly recorded.

• **Results** The concentration of DP was higher than that of DN in fresh litter, and the concentrations of both elements were determined by plant organs and species. Moreover, The DN and DP concentration was positively related to mean monthly temperature, while DN and DP storage was negatively correlated with mean monthly temperature and monthly precipitation. The storage of DN and DP was determined by litter biomass, which the order in litter from different plant organs was leaves>twigs>miscellaneous>flowers and fruits. The storage of DN and DP in leaves showed two peaks in April and October, but that in twigs and the miscellaneous showed only one peak in October.

• **Conclusion** Our results indicated that dissolved nitrogen (DN) is transferred and reabsorbed more than dissolved phosphorus (DP) before plant leaf senescence and other organs fall. Furthermore, DN and DP were associated with climate, plant organs and species in a subalpine forest on the eastern Tibetan Plateau.

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Contribution of the co-authors

Y.Z., F.W. and W.Y. conceived the project. All author conducted the field work. Y.Z. and J.Y. conducted the laboratory analyses. Y.Z. and J.Y. contributed to analysis and interpretation of the data. Z.Y. and F.W. wrote the manuscript. All authors critically reviewed the manuscript.

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1 Introduction

Fresh litter contains rich dissolved substances, such as dissolved organic carbon (DOC), dissolved nitrogen (DN), and dissolved phosphorus (DP) (Cleveland et al., 2004; Liao et al., 2015). Large amounts of dissolved organic and inorganic matter are released from fresh litter during the early period of litter decomposition (Berg, 2014). These dissolved substances provide the energy and nutrients for biological growth (Mcdowell et al., 2006) and contribute to the formation of soil organic matter (Hafner et al., 2005). Therefore, numerous studies have been performed in different ecosystems in recent decades to understand the dynamics of dissolved matters in litter, especially dissolved organic carbon (Cleveland et al., 2004; Mosher et al., 2015; Soong et al., 2014), but little research has been focused on DN and DP of fresh litter in the forest ecosystem.

The DN and DP are the dominant forms of total nitrogen and phosphorus in litter (Goller et al., 2006). The changes of nitrogen and phosphorus in fresh litter could be affected by multiple factors, such as the plant organs, the microenvironment, the plant species (Hafner et al., 2005; Uselman et al., 2009), and climate factors (including temperature and precipitation) (Michalzik et al., 2001). First of all, different plant organs vary in their formation and development (Xu et al., 2013), which causes the concentration of nitrogen and phosphorus are greatest in leaf litter (Chang et al., 2013). Secondly, affected by temperature and precipitation (Wang et al., 2016; Yang et al., 2016), the nitrogen and phosphorus in litter has obvious seasonal variation. Furthermore, precipitation easily promotes the release of dissolved substances from litter (Neff and Asner, 2001). Thirdly, the largest proportion of total annual biomass occurred in leaf biomass, but due to the influence of phenology, the biomass of flowers is larger in summer. In addition, different plant species have different biological and ecological characteristics (Simon and Jeannicolas, 2007), which directly impact the litter biomass and litter patterns, so they indirectly influence the DN and DP in fresh litter. In the current context of global climate change, there is considerable motivation to better understand these influencing factors. Therefore, we predicted that the DN and DP in fresh litter are not only determined by litter biomass, but also regulated by the plant organs and climate.

To test our hypothesis, we conducted a 1-year field experiment in a subalpine forest on the Tibetan Plateau, during which we measured the concentration and storage of DN and DP in organs and species of fresh litter. Plant species, organs, and climate factors affect DN and DP in fresh litter, but it is unclear which of these factors primarily determines the variation in these nutrients. In addition, it remains unclear

how the plant species, organs, and climate factor combine to affect DN and DP under field conditions. Therefore, this study addressed the following questions: (1) How do the concentrations and storage of DN and DP vary within a year? (2) How do species and organs of plants and climate factors affect the concentrations and storage of DN and DP in fresh litter? And which plays a decisive role?

2 Materials and methods

2.1 Site description

This study was conducted at the Long-term Research Station of Alpine Forest Ecosystem in the Bipenggou Valley of the Miyaluo Nature Reserve (102° 53'–102° 57' E, 31° 14'–31° 19' N; 2458–4619 m) in Li County, Sichuan Province, China. The reserve is a transitional area between the Tibetan Plateau and the Sichuan Basin, and the region is sensitive to global climate change and plays important roles in the conservation of both water and soil as well as in the protection of biodiversity (He et al., 2015). The mean annual temperature ranges from 2 to 4 °C, and the maximum and minimum temperatures are 23 °C (July) and – 18 °C (January), respectively. The mean annual precipitation is approximately 850 mm, and the maximum precipitation is about 134 mm (July), while the minimum precipitation is only 4 mm (December). The dominant arbor species include *Abies faxoniana* Rehder & E.H.Wilson, *Picea asperata* Mast, *Cerasus conadenia* (Koehne) Yu & Li, and *Cerasus pleiocerasus* (Koehne) Yu & Li. The understory shrubs are dominated by *Salix paraplesia* Schneid, and *Rosa omeiensis* Rolfe. The herbaceous plants mostly include *Cystopteris montana* (Lam.) Bernh. ex Desv, *Carex* Linn, and *Cyperus* Linn. The soil is considered a Cambisol (Ni et al., 2015), and the mineral soil is shallow due to frequent natural disasters and low temperatures (Liao et al., 2015).

2.2 Experimental design

Based on our previous survey of aspect, slope, and altitude (Fu et al., 2017), three replicate 50 m × 50 m sampling sites were established in a spruce-fir forest (2982–3020 m in altitude) at the end of July 2015. The sites were at least 50 m apart, and they were uniform in aspect and slope to avoid topographical variation. Twenty litter collectors were randomly placed in each sampling site with a minimum distance of 2 m between adjacent collectors to ensure that the litter samples in each plot were independent. The collectors were made of iron wire, advertising cloth and an ordinary black bag. Litter was collected in the black bag. The litter collectors were funnel-

shaped; that is, the diameters at the top and bottom were 1 and 0.15 m, respectively. Furthermore, they were supported by a wooden stick to facilitate collection.

Air temperature and precipitation were measured throughout the entire study period. iButton automatic recorders (iButton DS1923-F5, Maxim/Dallas Semiconductor, Sunnyvale, USA) were used to measure air temperature every 2 h. In each of the three sampling plots, an iButton was placed in a bag and hung on a shrub, and precipitation was measured by an atmospheric precipitation collector. The collected data are calculated and integrated to obtain monthly precipitation and mean monthly temperature. Monthly precipitation and mean monthly temperature recorded in this study are showed in Fig. 1.

2.3 Sample collection and analysis

Litter collection occurred from the end of August 2015 through the end of July 2016. To prevent the leaching due to rain and litter weight loss, the litter samples were collected twice a month during the peak litter periods and once a month during other months in this study. However, litter was collected only once between December 2015 and March 2016 because the snow depth (approximately 50 cm during this time) and frequent earthquakes made the sampling difficult. After sampling, we took the collected litters to the laboratory where these litters were air-dried. The collected litters were first manually classified according to the plant organs as leaves, twigs, flowers and fruits, and miscellaneous (including bark, lichens and epiphytic plants), and then leaf litter according to species. There were six major categories of foliar litter in this study, and these categories were divided into life form (arbor versus shrub) and leaf phenology (deciduous versus evergreen) according to the functional type of the plant (Yuan and Chen, 2009). The resulting classification of six species is shown in Table 1. Afterward, all samples were oven-dried at 65 °C for 72 h to a constant weight and weighed, which was mainly used to calculate storage. And the dried samples were subsequently shattered and sifted into subsamples.

Fig. 1 Monthly precipitation and mean monthly temperature in the research region from August 2015 to July 2016

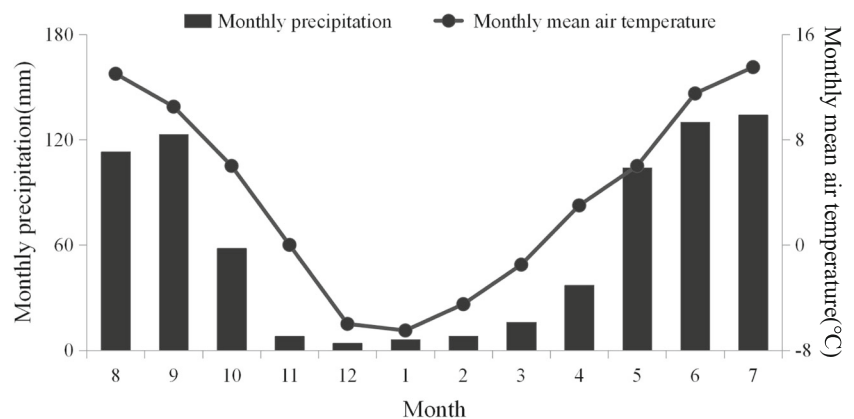


Table 1 Classification of six major categories of foliar litter in the study site of the Tibetan Plateau

Plant species	Life form		Leaf phenology	
	Arboreal	Shrub	Evergreen	Deciduous
<i>A. faxoniana</i>	√	–	√	–
<i>P. asperata</i>	√	–	√	–
<i>C. conadenia</i>	√	–	–	√
<i>C. pleiocerasus</i>	√	–	–	√
<i>S. paraplesia</i>	–	√	–	√
<i>R. omeiensis</i>	–	√	–	√

Subsamples were used to determine the concentrations of DN and DP. We first accurately weighed 0.5 g subsamples and then placed them in 150 ml conical flasks, to which we added 50 ml of deionized water. To fully dissolve the subsamples into the deionized water, the conical flasks were centrifuged for 30 min at 25 °C, and then we removed the supernatants by filtering the solutions through 0.45 μm membranes (Uelman et al., 2009). The solutions were immediately refrigerated at 4 °C. To ensure the accuracy of the experimental data, all analyses were completed within 1 week after sample collection. The DN and DP concentrations of the extracted solutions were determined by the Kjeldahl (KDN, Top Ltd., Zhejiang, China) and molybdenum antimony colorimetric methods (TU-1901, Puxi Ltd., Beijing, China), respectively. All analyses were performed in triplicate.

2.4 Statistical analyses and calculations

The concentrations of DN and DP were determined, and the storage of DN and DP were expressed by *S*, which was calculated as follows (Huang et al., 2008):

$$S = C \times M.$$

In this equation, *C* is the concentration of DN or DP, and *M* is the litter biomass per unit area (Annex Table 5). This

experiment evaluated the concentrations and storage of DN and DP in fresh litter originating from the arbor and shrub layers; fresh litter from the herb layer was not included.

All statistical analyses were performed using SPSS software (SPSS 20.0 for Windows; SPSS Inc., Chicago, IL, USA), and figures were constructed using Excel 2010 and Origin 8.5 software. According to the W-test and Levene test, all experimental data are in conformity with the necessary assumption of normality and homogeneity of variance. Repeated measures ANOVA was used to test the effects of plant organs, species and their interaction on concentration and storage of DN and DP. Correlation analysis was used to analyze the relationships between the climate (monthly precipitation and mean monthly temperature) and the concentrations and storage of DN and DP.

Linear regression analyses among DN and DP concentrations and plant biomass were performed to predict DN and DP storage.

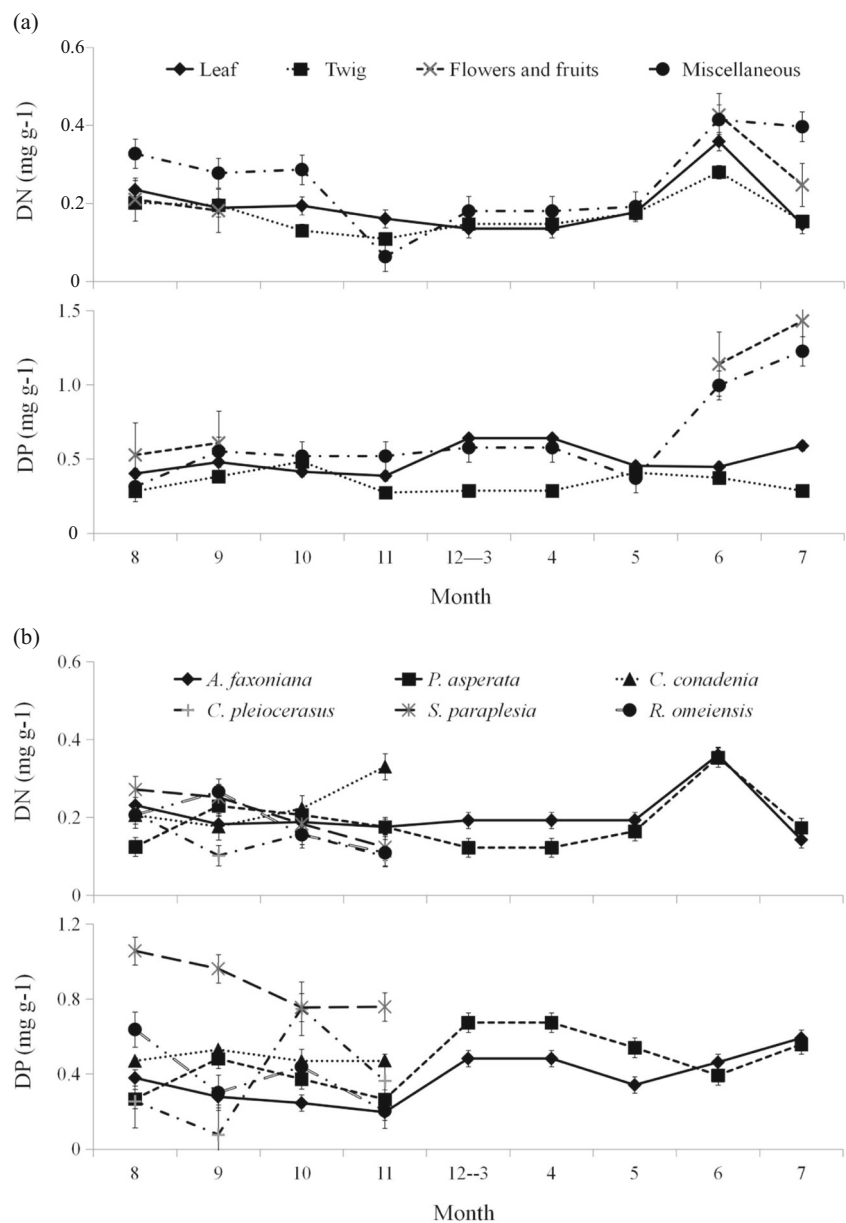
Data availability statement All the raw data used the present study are available from the authors upon reasonable request and with permission of the corresponding author F.W.

3 Results

3.1 Concentrations of DN and DP

There was a great difference in the dynamic change of DN and DP concentration. The DN concentration in fresh litter

Fig. 2 Concentration of dissolved nitrogen (DN) and dissolved phosphorus (DP) in the study area from August 2015 to July 2016. **a** Plant organs. **b** Plant species (four deciduous plants had no leaves fall from December 2015 to July 2016)



showed a relatively narrow range, from 0.06 to 0.42 mg g⁻¹, whereas the DP concentration ranged from 0.09 to 1.43 mg g⁻¹ (Fig. 2). In general, the DN concentration was lower than that of DP in fresh litter. The DN concentration was the largest in miscellaneous except during November 2015; however, the DP concentration of flowers and fruits was the greatest (Fig. 2a). Furthermore, the DN concentrations of all organs were highest in June (Fig. 2a).

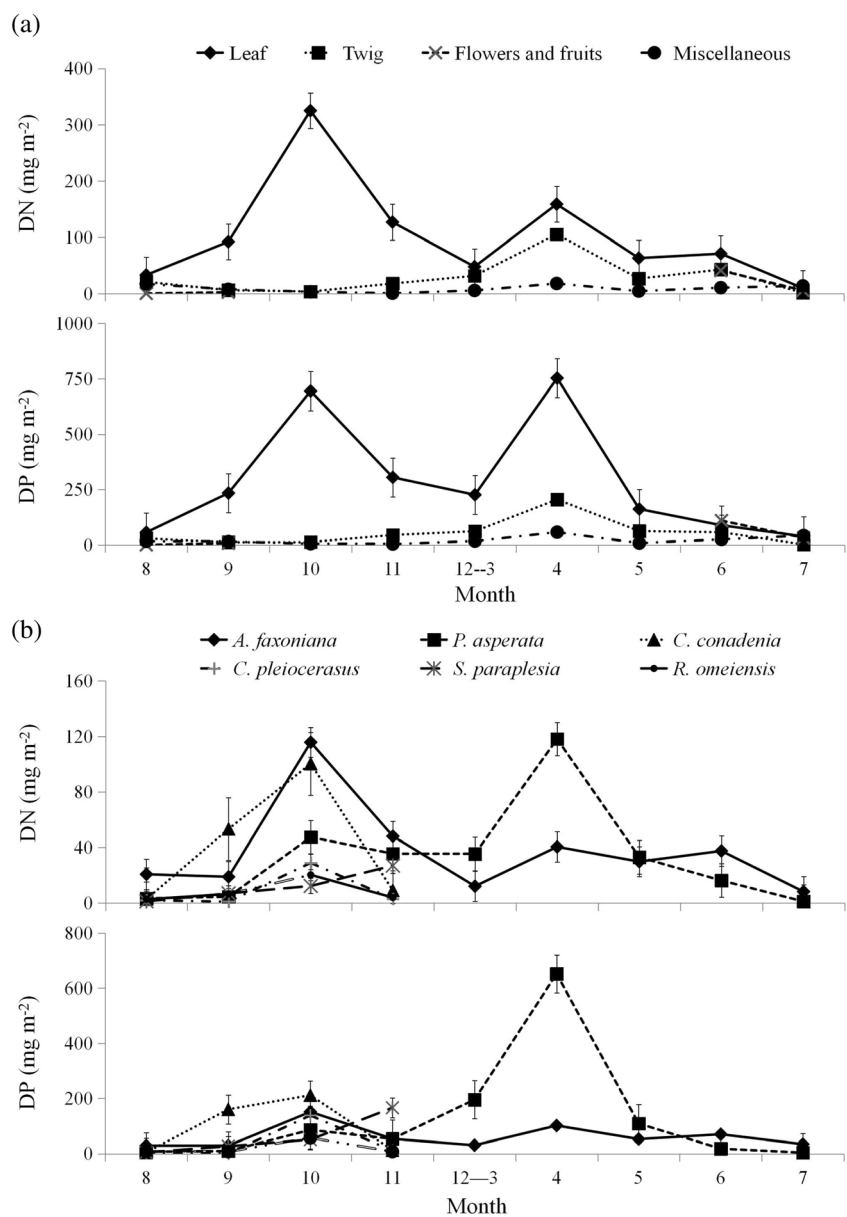
Regarding the leaf litter, the DN concentrations of evergreen plants (*A. faxoniana* and *P. asperata*) were highest in June, but for the deciduous plants, the DN concentration of *C. conadenia* was significantly higher than that of other deciduous plants in November (Fig. 2b). However, the DP concentration completely differed from that of DN. Of all the leaf

litters, the DP concentration in *S. paraplesia* was greatest. Furthermore, the DP concentration of *A. faxoniana* was highest in July, but the DP concentration of *P. asperata* was largest in the snow cover period (from December to April of the following year) (Fig. 2b). For deciduous plants, the DP concentrations were highest in *S. paraplesia* and *R. omeiensis* in August, whereas it was highest in *C. pleiocerasus* in October (Fig. 2b).

3.2 Storage of DN and DP

The annual total storage of DN in the study was 13.1 kg hm⁻² year⁻¹, and the greatest proportion was observed in leaves (70.7%), followed by twigs (19.5%), miscellaneous

Fig. 3 Storage (concentration × biomass) of dissolved nitrogen (DN) and dissolved phosphorus (DP) in the study area from August 2015 to July 2016. **a** Plant organs. **b** Plant species (four deciduous plants had no leaves fall from December 2015 to July 2016)



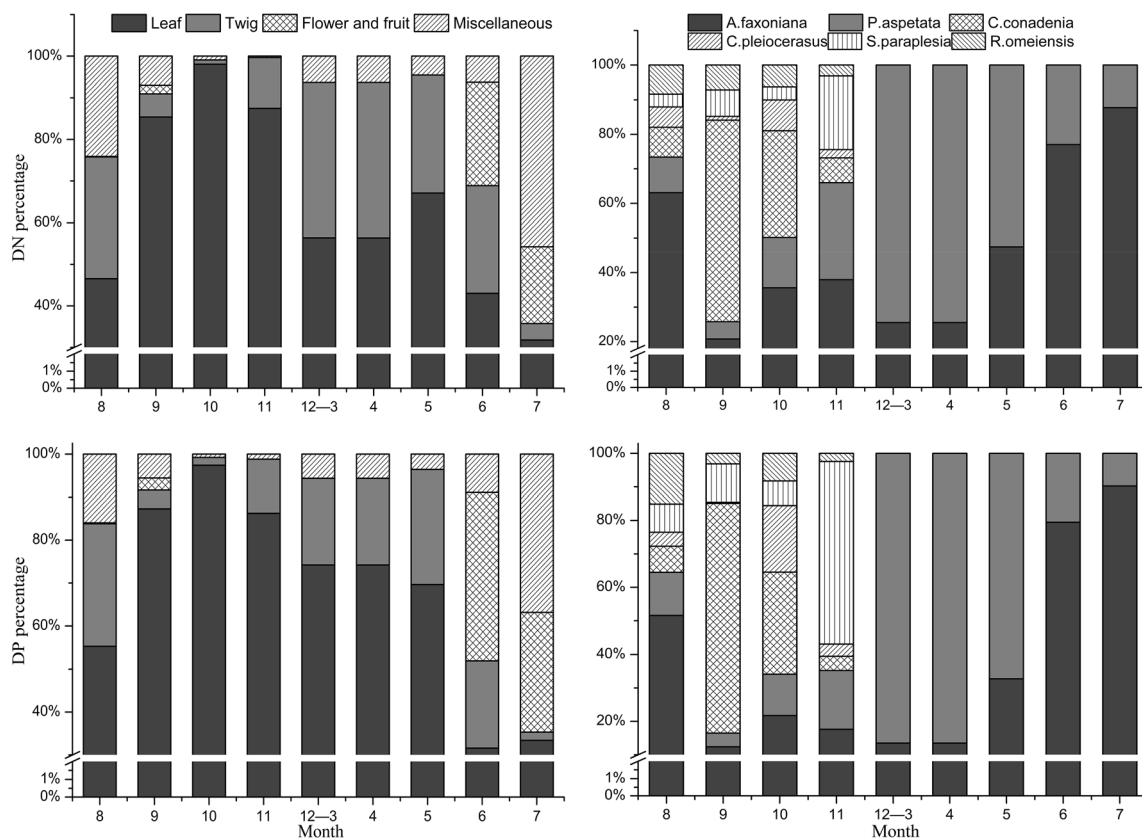


Fig. 4 Percentages of dissolved nitrogen (DN) and dissolved phosphorus (DP) storage (concentration × biomass) in the study area from August 2015 to July 2016 (four deciduous plants had no leaves fall from December 2015 to July 2016)

(6.1%), and flowers and fruits (3.7%). The annual total storage of DP was 33.8 kg hm⁻² year⁻¹, and the proportions were 73.6% in leaves, 16.6% in twigs, 5.5% in miscellaneous, and 4.3% in flowers and fruits. The distribution patterns of the total annual storage of DP and DN in litter from different plant organs were the same: leaves>twigs>miscellaneous>flowers and fruits.

The DN and DP storage in leaf litter had two peaks in April and October, and the DN storage in October was significantly higher than that in April (Fig. 3a). The largest proportions of DN and DP storage in leaf litter occurred in October

(approximately 97% of monthly total storage), whereas the smallest proportion were observed in summer (June and July) (Fig. 4). However, the DN and DP storage in twigs and miscellaneous exhibited a single peak in April, and other months have a very small fluctuation (Fig. 3a). In twigs, the greatest percentage of DN storage occurred in December to April and accounted for 37% of the monthly total storage, whereas the greatest proportion of DP storage was observed in August (Fig. 4). The largest proportion of DN and DP storage in miscellaneous was in June, while that of in flowers and fruits occurred in July (Fig. 4).

Table 2 Effect of the plant species and organs and their interaction on the concentration and storage (concentration × biomass) of dissolved nitrogen (DN) and phosphorus (DP) analyzed by repeated measures ANOVA (*p* < 0.05). *F* values are showed. * indicates *p* < 0.05, ** indicates *p* < 0.01, *** indicates *p* < 0.001

Factor	DN concentration	DP concentration	DN storage	DP storage
Time	189.534***	150.583***	4.447**	5.501**
Organs	65.888***	268.466***	2.276	0.396
Species	192.854***	167.018***	7.431**	6.34**
Organs × species	97.889***	19.655***	14.302**	11.501***
Time × organs	169.154***	173.627***	1.583	1.018
Time × species	44.655***	60.053***	1.482	4.464**
Time × organs × species	220.343***	200.552***	4.7**	10.141***

Table 3 The relationship between the concentration and storage (concentration \times biomass) of dissolved nitrogen (DN) and dissolved phosphorus (DP) and the climate (monthly precipitation and meanmonthly temperature). Values show Person correlation coefficient. * indicates $p < 0.05$, and ** indicates $p < 0.01$

	DN concentration	DP concentration	DN storage	DP storage
Mean monthly temperature	0.476**	0.311*	-0.302*	-0.281*
Monthly precipitation	0.479	0.271	-0.287*	-0.276*

Across foliar litters, the storage of DN and DP in evergreen plants peaked twice in April and October (Fig. 3b). The DN and DP storage in *A. faxoniana* was greatest in October, but the greatest proportion occurred in July (approximately 90%) (Fig. 3b, Fig. 4). Similarly, the storage of DN and DP in *P. asperata* was greatest in April, whereas the largest proportion was observed in the snow cover period (from December to April of the following year) (Fig. 3b, Fig. 4). In contrast, the storage of DN and DP in deciduous plants exhibited one peak in October. The storage of DN and DP in *Cerasus* and *R. omeiensis* was the greatest in October, whereas that in *S. paraplesia* was largest in November (Fig. 3b). However, the largest proportions of DN and DP storage in *C. conadenia* occurred in September, whereas that in *R. omeiensis* occurred in August and more than half of the monthly total storage (Fig. 4). Overall, the storage of DN and DP in deciduous plants was mainly concentrated in autumn.

3.3 Influence of climate, plant organs and species on DN and DP

Repeated measures ANOVA showed that plant organs and species and their interaction had an extremely significant influence on DN and DP concentration. Plant species had an extremely significant influence on DN and DP storage, but plant organs had no effect on DN and DP storage. Moreover, the interaction of plant organs and species also had an extremely significant influence on DN and DP storage (Table 2). Moreover, the DN concentration was positively correlated with the mean monthly temperature and monthly

precipitation, while DP concentration was only positively correlated with the mean monthly temperature (Table 3). The storage of DN and DP was negatively correlated with the monthly precipitation and mean monthly temperature (Table 3). We also found that the storage of DN and DP was extremely significant and positively related to litter biomass, while not related to concentration (Tables 4). Furthermore, DN storage was significantly positively related to DP storage.

4 Discussion

We observed that, in general, the concentration of DP was larger than that of DN, and this result is consistent with those of Xu et al. (2014). However, our results differed from those of other authors (Oheimb et al., 2010; Han et al., 2005) who found that the concentration of total nitrogen was greater than total phosphorus in litter. Compared to total nitrogen and phosphorus, DN and DP are easily diminished due to the effects of climate, microbes and litter quality (Adair et al., 2008; Preston et al., 2009), and plants must transfer more nitrogen to reduce transpiration rates (Yang et al., 2016). Furthermore, Oyarzabal et al. (2008) found that nitrogen resorption efficiency decreases with increasing temperature. In our research area, the temperature is low and nitrogen resorption efficiency will increase; thus, the concentration of DN was less than that of DP in fresh litter. In other words, plants absorb more DN before plant leaf senescence and other organs fall. Our finding is also supported by results from common studies (Yuan and Chen, 2009a), which showed that low-temperature sites, plant species are highly dependent on internal nitrogen cycling processes.

The study also indicated that fresh litter contains a large amount of DN and DP. Dissolved matters in litter are the major nutrient sources for soil organisms (Cleveland et al., 2004). Fresh litter falls to the ground, and the DN and DP in litter are released into the soil by natural decomposition, which increase soil organic matter. We further demonstrated that the concentrations and storage of DN and DP had clear seasonal dynamics. The concentration of DN and DP in the fresh litter increased with increasing mean monthly

Table 4 Linear regression analysis ($p < 0.05$) between the concentrations and storage (concentration \times biomass) of dissolved nitrogen (DN) and dissolved phosphorus (DP)

Y	X	Equation	R^2	P
DN storage	DP storage	$Y = 0.235X + 9.229$	0.633	<0.001
DN storage	DN content	$Y = -17.774X + 27.309$	0.0026	0.722
DN storage	Biomass	$Y = 0.148X + 4.542$	0.899	<0.001
DP storage	DP content	$Y = 41.844X + 38.804$	0.015	0.391
DP storage	Biomass	$Y = 0.473X + 0.412$	0.795	<0.001

temperature, which is contrary to studies of Reich and Oleksyn (2004) who found that the concentration of total nitrogen and phosphorus decrease with the increasing temperature through a study of 1280 plants around the world. There are thicker snow cover and lower temperatures in our research areas. Low temperatures stimulate plants to synthesize abscisic acid (Alcázar et al., 2010), which can influence plant chemistry and vegetative composition (Yuan and Chen, 2009b). Each of them can influence DN and DP concentration. In this research, the plant organs and species affected concentration of DN and DP, but it differs from the results reported by Shaunam et al. (2009), and they thought individual species were unlikely to have a significant effect on the changes in the concentration of DN and DP. This may be due to the different structural compositions of plant organs and species, including the complex the carbon to nitrogen stoichiometry and carbon to phosphorus stoichiometry (Soong et al., 2014). In addition, the order of DN and DP storage in litter form different plant organs was leaves > twigs > miscellaneous > flowers and fruits. It is consistent with litter biomass, which follows the order of leaves > twigs > miscellaneous (Li et al., 2013). Thus, the storage of DN and DP was related to litter biomass, which is consistent with previous studies (Shaunam et al., 2009). Moreover, the storage of DN and DP was negatively correlated with the monthly precipitation and mean monthly temperature, which is consistent with our hypothesis.

The DN and DP storage in the leaf litter initially increased, then decreased and finally increased again within a year. The two peaks in DN and DP storage occurred in spring (April) and autumn (October), whereas the twig and miscellaneous showed one peak in April, which is consistent with the seasonally dynamic model of forest litter biomass (Yuan et al., 2010). In spring (April to May), the temperature rises and precipitation increases, and many plants enter their growing season. Deciduous plants begin to sprout and grow new shoots. Although evergreen plant leaves gradually wither, most exhibits relatively concentrated leaf renewal during this time. Evergreen plants need to transfer large amounts of nutrients from old leaves to new leaves to maintain plant growth (Güsewell, 2004), so the concentrations of DN and DP in the leaf litter are small but the biomass is high when this first peak. Therefore, the storage of DN and DP in leaf litter exhibited its first peak. However, the factors that affect the biomass of plant organs are different. The leaf litter is mostly affected by temperature and plant type; twig litters are mostly affected by moisture (Zhang et al., 2008). During the growing season, the snow is melting and precipitation is increasing, which leads to the increase of the twig litter biomass, so the storage of DN and DP in twigs appeared to peak in April. In summer (June to August), plant leaves are at the stage of growth, they require to store a great deal of

nutrients to meet their needs; thus, the concentrations of DN and DP are highest in leaves at this period. In June, due to the influence of phenology, most flowers begin to fade, and the biomass of flower litters are increasing. Thus, the storage of DN and DP in flowers and fruits are greater than other months. In autumn (September to November), the temperature began to decrease. Furthermore, low temperatures stimulate plants to synthesize abscisic acid (Alcázar et al., 2010), which promotes the dropping of leaves to reduce the consumption of nutrients and water (Yuan et al., 2010). At this point, another peak of the leaf litter appeared, which mostly consisted of the leaves of deciduous plants. This result also illustrated that the leaf litter biomass of deciduous plants exhibits one peak, whereas that of evergreen plants exhibits two peaks. In winter (December to March), the leaves of the deciduous plants have fallen off, the leaf litter is mainly composed of evergreen plants.

5 Conclusion

We investigated the DN and DP in the fresh litter of a subalpine forest on the eastern Tibetan Plateau in China. Our results indicated that DN is transferred and reabsorbed more than DP before plant leaf senescence and other organs fall, and were associated with climate such as monthly precipitation and mean monthly temperature, plant organs and species in a subalpine forest on the eastern Tibetan Plateau. Although 1 year of research on DN and DP in fresh litter is limited to understand the nutrient cycling in subalpine forests, these patterns have important implications for our understanding the nitrogen and phosphorus cycle of terrestrial ecosystem and nutrient transfer, and offer basic data for the development of modeling tools useful at regional to global scales.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Annex

Annex Table 5 Biomass of per unit area in litter (g m^{-2}) of the different plant species and organs monthly collected in the study site

Months	Leaves						Twigs	Flowers and fruits	Miscellaneous
	<i>A. faxoniana</i>	<i>P. asperata</i>	<i>C. conadenia</i>	<i>C. pleiocerasus</i>	<i>S. paraplesia</i>	<i>R. omeiensis</i>			
8	76.59	27.26	9.33	9.26	4.48	13.34	102.87	0.42	52.02
9	103.91	19.96	302.58	9.77	27.77	24.54	30.61	12.36	26.99
10	616.75	230.20	450.73	184.52	67.30	130.92	26.87	–	10.64
11	274.27	202.63	27.45	30.60	218.83	36.51	162.72	–	8.04
12–3	63.15	289.89	–	–	–	–	214.69	–	29.78
4	210.50	966.32	–	–	–	–	715.96	–	99.24
5	154.77	201.34	–	–	–	–	152.28	–	22.32
6	151.50	46.00	–	–	–	–	152.19	95.89	24.98
7	57.85	6.65	–	–	–	–	7.61	22.13	34.13

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